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Korrespondenzadresse:
asmtbb@gmail.com

[1]) Department of Food Engineering, Agriculture Faculty, Selçuk University, 42050, Konya, Turkey; [2]) Department of Food Processing, Çumra Vocational School, Selcuk University, 42400, Çumra/Konya, Turkey; [3]) Department of Food Engineering, Agriculture Faculty, Selçuk University, 42050, Konya, Turkey; [4]) Department of Food Engineering, Agriculture Faculty, Selçuk University, 42050, Konya, Turkey

Ultrasound application and electrolyzed water combination improve the microbial quality and textural parameters of chicken breast meats

Die Kombination aus Ultraschallanwendung und elektrolysiertem Wasser verbessert die mikrobiologische Qualität und die texturellen Parameter von Hähnchenbrustfleisch

Ali Samet Babaoğlu[1]), Hatice Berna Poçan[2]), Talha Demirci[3]), Mustafa Karakaya[4])

Summary

The combined effects of ultrasound application and dipping in electrolyzed water on quality and shelf life of the refrigerated chicken breast meats were investigated during refrigerated storage (4 °C) for 7 days. The chicken breast meats were dipped in tap water (TW – pH 8.0), acidic electrolyzed water (AW – pH 2.1) and basic electrolyzed water (BW – pH 11.4) and then treated with different durations of ultrasound application as 0, 15 and 30 min (24 kHz and power intensity of 400 W/cm^2). The samples were immersed in the waters at room temperature for total 30 min. Microbiological, physicochemical (pH, WHC, TBA, color), sensory and textural parameters (MORSF and MORSE) were determined for 1, 4 and 7 days. While AW treatment increased the TBA values, ultrasound application significantly ($p<0.05$) inhibited lipid oxidation. The lowest psychrophilic bacteria, *Enterobacteriaceae* and *Salmonella* spp.counts were observed in the group of U1-AW during the storage days ($p<0.05$). The group of U3-AW had the lowest counts of *Pseudomonas* spp. on day 7 ($p<0.05$). The 30 min ultrasound application increased the tenderness of the samples ($p<0.05$). The combination of ultrasound and acidic electrolyzed water treatment could be recommended owing to its reduction effect on microbial counts and owing to the fact that it has no negative changes regarding lipid oxidation and color parameters of the chicken breast meats.

Keywords: Chicken breast meat, Electrolyzed water, Microbial quality, Ultrasound, Texture

Introduction

The food industry deals with emerging technologies that include electrolyzed water treatment, ultrasound application, high hydrostatic pressure, shock waves, high-intensity light, carbon dioxide and gas plasma application due to consumers' demands for foods in recent years (Guan and Hoover, 2005). The growing demand for safe, minimally processed food and the disadvantages of the traditional chemicals and the heat-based methods of microbial decontamination mean that new technologies are emerging for high quality, effective decontamination and high quality food (Cheng et al., 2012). In the meat industry, microbial contamination is an important topic because it can decrease the shelf life and it poses a risk to food safety (Patsias et al., 2006; Petrou et al., 2012).

Electrolyzed water (EW) is used due to its antimicrobial effect on different spoilage and pathogen microorganisms (Gómez-López et al., 2015; Han et al., 2018). Electrolyzed water is seperated into 3 categories as acidic electrolyzed water, slightly acidic electrolyzed water and basic electrolyzed water. The basic electrolyzed solution (pH> 10 and ORP <- 800 mV) is formed by the cathode. It has a strong reduction potential. The electrolyzed acid solution (pH <2.7 and ORP> 1100 mV) is generated by the anode. It has a strong oxidation potential and can be used as bacterial disinfectant (Kim et al., 2000; Len et al., 2000; Hsu, 2005). Slightly acidic electrolyzed water (pH value of 6.0-6.5 and ORP 800-900 mV) can be obtained by electrolysis without a membrane or with a membrane and a mixture of acidic and basic electrolyzed water (Guentzel et al., 2008; White, 2010).

Researchers have reported that electrolyzed water applications have important effects on the inactivation of *E. coli* O157:H7, *S. enteritidis,* and *L. monocytogenes* (Venkitanarayanan et al., 1999; Kim et al., 2000). EW treatments in poultry meat have been studied and these studies have shown that microbial contamination was effectively reduced (Park et al., 2002; Kim et al., 2005; Huang et al., 2008; Shimamura et al., 2016; Wang et al., 2018; Cichoski et al., 2019; Hernández-Pimentel et al., 2020).

One of the other novel processing technologies is ultrasound. Ultrasound technology has been used in food industry such as marination, meat tenderizing, inactivation of microorganisms and enzymes, crystallization, freezing, drying, degassing, filtration, emulsification and homogenization (Chemat et al., 2011). In meat and meat products, ultrasound has been utilized to improve the microbiological content, physicochemical and sensory characteristics (Alarcón-Rojo et al., 2015; Flores et al., 2018; Zou et al., 2019; Shi et al., 2020; Xiong et al., 2020; Zhang et al., 2020).

In a study, it has been reported that the *Salmonella* population in the skin of broilers was diminished with an ultrasound application at 20 kHz in 30 min (Dolatowski et al., 2007). Some researches have shown that the density of conventional heat treatments can be decreased by 50% when combined with power ultrasound. Some authors have reported that ultrasound was applied to a chicken skin surface in water and in 1% aqueous lactic acid. After this application (40 kHz and 2.5 Wcm^{-2} for 3 or 6 min) the gram-negative bacteria on the surface of the chicken skin were eliminated (Kordowska-Wiater and Stasiak, 2011). When fresh beef was treated with power ultrasound application (40 kHz and 60 W/cm^2 for 60 and 90 min), it was observed that coliforms and psychrofilic bacteria load decreased. As the treatment duration of the meat prolonged, the load of microorganisms decreased further (Caraveo et al., 2015). Studies in meat technology show the changes in meat tenderization and inactivation of the microorganisms due to ultrasound. Furthermore, the combination of ultrasound and a disinfectant agent may increase the effect of the microbial reduction in foods (Alarcon-Rojo et al., 2018; Cichoski et al., 2019).

Hence, the objective of this study was to determine the effects of acidic and alcaline electrolyzed water and ultrasound application in different treatment durations (15 and 30 min) on microbial, textural and some physicochemical properties of the chicken breast meats and to observe the differences in quality parameters throughout the storage periods.

Materials and methods

Materials

Boneless, skinless broiler chicken breast meats were obtained from a local market in Konya.

Production of electrolyzed water

In this study, tap water (pH 8.0) was used as the control group and acidic and basic electrolyzed water were prepared from the tap water. Electrolyzed waters were obtained by using a device (ЭКОВОД-6 ЖЕМЧУГ (Kiev, Ukraine)). The device is composed of three compartments and these compartments are divided equally by 2 membranes. Pods were filled with tap water and 0.3 % salt (NaCl) was added, then the device was switched on. When the ion (H^+ and OH^-) transition through the membranes was complete we obtained acidic water (pH 2.1) and basic water (pH 11.4).

Ultrasound application

Ultrasound application (sonication) was performed with an ultrasound system (Hielscher, UP400S model, GmbH, Teltow, Germany) which had an operating frequency of 24 kHz, a power density of 400 W/cm^2 and an amplitude level of 100 %. Sonication was applied using a probe which had a vibrating horn diameter of 40 mm. The probe was directly immersed in the waters (acidic, basic and tap water) including chicken breast samples for ultrasound application. The probe did not touch the samples. Each sample was exposed to sonication for different periods of time (15 and 30 min).

Experimental Design

In this study, the chicken breast samples were cut into equal parts of approximately 30±0.5 g (5 x 2.5 x 2.5) and placed randomly in nine different polypropylene boxes and each had ten samples. The boxes were grouped three by three and each group was treated with three different waters which were tap water, acidic electrolyzed water and basic electrolyzed water. Each sample remained in the water for total 30 min and then all of the samples were treated with different ultrasound application durations, which were respectively 0, 15 and 30 min. The control group was kept in tap water without ultrasound application (0 min). After ultrasound application, water was removed from each box and samples were stored in the polypropylene boxes for up to 7 days at 4°C in a refrigerator. Nine different sample groups in total were analyzed on the 1^{st}, 4^{th} and 7^{th} days. The experimental design of the study is shown in Table 1.

TABLE 1: *Experimental design of the study.*

Water Treatment	Duration of ultrasound treatment (minutes) + water treatment (minutes)	Storage periods (days)
Tap water (control) (TW)	0 (U0) + 30 15 (U1) + 15 30 (U3) + 0	1st, 4th, 7th
Acidic electro-lyzed water (AW)	0 (U0) + 30 15 (U1) + 15 30 (U3) + 0	1st, 4th, 7th
Basic electro-lyzed water (BW)	0 (U0) + 30 15 (U1) + 15 30 (U3) + 0	1st, 4th, 7th

The samples were kept in water for 30 min in total. Sample codes: Water Treatments; TW: tap water, AW: acidic water, BW: basic water. Ultrasound Applications; U0: 0 min, U1: 15 min, U3: 30 min.

Determination of proximate composition

Water, total protein, total fat (ether extraction) and total ash contents of the chicken breasts were determined according to AOAC (2000). The water, total protein, total fat and total ash contents of the samples were expressed in percentages (%). The proximate composition of chicken breast meat used in the study was determined.

Determination of water holding capacity and pH

The pH values of the samples were measured with a pH meter (WTW 315 i set model, Weilheim, Germany) according to AOAC (2000).

The method reported by Wardlaw et al. (1973) was used to determine the water holding capacity (WHC) of the chicken breast meats. The samples (8 g) and 12 ml 0.6 M NaCl solution were put into a tube and shaken. The tubes stayed in a water bath (5°C) for 15 min, and the tubes were centrifuged (4°C) at 10.000 rpm for 15 min. The supernatant was obtained to determine the WHC (%) of the chicken breast meats.

Thiobarbituric acid (TBA)

The method described by Ockerman (1985) was used to determine the extent of oxidative rancidity (TBA value) of the samples in 1, 4 and 7 days after processing. The absorbance was read at 538 nm (UV-160 A, Spectrophotometer, Shimadzu, Tokyo, Japan). The TBA numbers were expressed as milligrams malonaldehyde per kilogram sample (mg MA/kg sample).

Microbiological analysis

Psychrotrophic bacteria was enumerated by using Plate Count Agar (PCA, Merck, Darmstadt, Germany) and incubated at 7°C for 8 days (Cousin et al., 2001). *Pseudomonas* spp. were counted by inoculation of *Pseudomonas* agar base (Oxoid) prepared with CN *Pseudomonas* supplement (Oxoid) and incubation at 25°C for 72h (Blanco et al., 2017). Violet Red Bile Glucose Agar (VRBG, Merck, Darmstadt, Germany) and *Salmonella-Shigella* Agar (SS, Merck, Darmstadt, Germany) plates were incubated at 37°C for 24h for the enumeration of *Enterobacteriaceae* and *Salmonella-Shigella* (Shiningeni et al., 2019). H2S producing colonies that have colorless, transparent with a black center morphology were recorded *Salmonella* spp. Results were expressed as colony-forming units (CFU Log10/g sample). The results were calculated as a means of log colony forming units per g sample (log CFU/g sample) of the chicken breast meat.

Textural analysis

Textural analysis was performed on both raw and cooked samples on the 1st day. Textural analysis of the raw samples which were kept in the refrigerator (4°C) was carried out after the raw samples reached at room temperature. For cooking process, the chicken breast meats were individually wrapped in aluminium foil and cooked at 180±2°C in the oven (Nüve-FN120) until the internal end-point temperature of 78°C was reached using a thermometer (Digitale Bratengabel – TCM). After the cooking process, the samples were cooled down to room temperature and then textural analysis was carried out. Textural properties of the chicken breast meats were analyzed using Meullenet Owens Razor Shear (MORS) method (Cavitt et al., 2004). Texture Analyzer (Model TA-XT-plus, Texture Technologies Corp, Hamilton, MA) with a 50-kg load cell was used for the determination of textural parameters of the samples. Meullenet Owens Razor Shear force (MORSF) and energy (MORSE) were determined by means of the MORS blade which has 0.5 mm thickness, 30 mm height and 8.9 mm wideness. As mentioned in the experimental design (2.4) section, texture analysis was performed on the chicken breast samples which were cut into equal parts (5 x 2.5 x 2.5 cm). Meullenet Owens Razor Shear force (MORSF) and the energy (MORSE) of the samples were expressed as N and N.mm, respectively.

Color measurements

Color measurements were performed according to Hunt et al. (1991). Chroma meter CR-400 (Konica Minolta, Inc., Osaka, Japan) with illuminant D65, 2° observer, Diffuse/O mode was used for color measurement. *L**, *a** and *b** parameters of the samples were determined. After the samples were taken out of the polypropylene boxes, their color properties were measured. The measurements were carried out 3 times on different parts of the chicken breasts.

Sensory analysis

A sensory panel composed of eleven semi-trained panelists carried out the sensory evaluations of the chicken breast meat samples. The breast fillet samples were cooked in aluminum foil at 180°C for 20 min. The samples were coded with three digit numbers and nine chicken breast samples were served to the panelists randomly in one session. Each sample was evaluated in terms of color, odor, tenderness and overall acceptance using 9-point hedonic scale (9: like extremely; 1:dislike extremely) (Gökalp et al., 1999).

Statistical analysis

A completely randomized design was employed and the experiment was performed in duplicate with triplicate sampling. Collected data was statistically analyzed using MINITAB for Windows Release 16. When significant difference was found ($p < 0.05$), Turkey Test was used to detect the differences between the values.

Results and discussion

Proximate composition of chicken breast meat

The means of the investigated chicken breast meat characteristics were as follows: 5.80 for pH value, 22.91 % protein, 74.84 % water, 1.05 % fat and 0.89 % ash. Similar values were reported by Lonergan et al. (2003), Li et al. (2015), (Tengilimoglu-Metin and Kizil, 2017) and (Da Silva-Buzanello et al., 2019) who reported the proximate composition of broiler breast meat.

WHC and pH measurements

The pH values of the samples are presented in Table 2. Mean pH values of the samples ranged from 5.49 to 6.03. Ultrasound application and electrolyzed water treatment had no significant effect on the pH values of the samples (p>0.05). During the storage, it is expected of pH to increase due to the alkaline components produced by proteolytic reactions (Bechet et al., 2005). However, in this study, differences of the pH values of the samples were not significant during storage periods. This situation could be attributed to the formation of acidic components produced by lactic acid bacteria that prevent the pH values from increasing during the storage (Pothakos et al., 2015). Similarly, Dolatowski et al. (2000) and Honikel et al. (1998) found that there was no significant influence of ultrasound treatment on the pH of the meat samples. In addition, in agreement with the results in the present study, Carrillo-Lopez et al. (2019) and Stadnik et al. (2008) did not find an effect of ultrasound application on the pH values of the meat samples. On the other hand, Cichoski et al. (2019) reported that there were no statistically significant differences in the pH values of the chicken breast meat samples based on electrolyzed water treatments. Similarly, Athayde et al. (2017) reported that all types of electrolyzed water and storage did not affect the pH of pork meat.

Figure 1 indicates the effect of ultrasound and electrolyzed water treatments on the WHC values of the samples during the 7 day storage. Mean WHC values of the samples were between 37.00 and 89.00%. Electrolyzed water treatment did not affect the WHC values of samples (p>0.05). Ultrasound application, storage and electrolyzed water x ultrasound interaction showed significant effect on WHC of the samples (p<0.05). 15 (U1) and 30 (U3) min ultrasound application increased the WHC of the samples when compared to U0. The highest WHC values of the samples were determined on the 4th day. U0-AW had the lowest mean WHC value, whereas U1-BW and U3-BW had the highest mean values. It has been reported that ultrasound causes release of the myofibrillar proteins, which are responsible for binding properties of the meat such as the water holding capacity (McClements, 1995). The WHC results of our study are in agreement with those of Pohlman et al. (1997), Dolatowski et al. (2007) and Chang et al. (2015). They observed an icrease in the samples' water holding capacity, too.

TABLE 2: *Mean pH values of samples.*

Treatments		pH Day 1	Day 4	Day 7
U0	TW	5.87±0.05^{Aa}	5.82±0.16^{Aa}	5.99±0.04^{Aa}
	AW	5.69±0.13^{Aa}	5.49±0.06^{Aa}	5.64±0.01^{Aa}
	BW	5.81±0.01^{Aa}	5.85±0.13^{Aa}	5.83±0.03^{Aa}
U1	TW	5.68±0.27^{Aa}	5.74±0.33^{Aa}	5.82±0.35^{Aa}
	AW	5.71±0.04^{Aa}	5.62±0.03^{Aa}	5.71±0.08^{Aa}
	BW	5.97±0.13^{Aa}	5.99±0.07^{Aa}	6.03±0.06^{Aa}
U3	TW	5.80±0.02^{Aa}	5.81±0.04^{Aa}	5.97±0.19^{Aa}
	AW	5.76±0.06^{Aa}	5.74±0.01^{Aa}	5.77±0.06^{Aa}
	BW	5.83±0.06^{Aa}	5.80±0.08^{Aa}	5.97±0.16^{Aa}

Within the same row, values with different uppercase superscript letters indicate significant differences (p < 0.05) for each different analyzes. Within the same column, values with different lowercase superscript letters indicate significant differences (p < 0.05). Values are the mean of three replicates in two independent experiments ± standard error. Water Treatments; TW: tap water, AW: acidic water, BW: basic water. Ultrasound Applications; U0: 0 min, U1: 15 min, U3: 30 min.

TBA

Figure 2 shows that the effect of ultrasound and electrolyzed water treatments on the mean TBA values (mg MA/kg) of the chicken breast meat samples. The results indi-

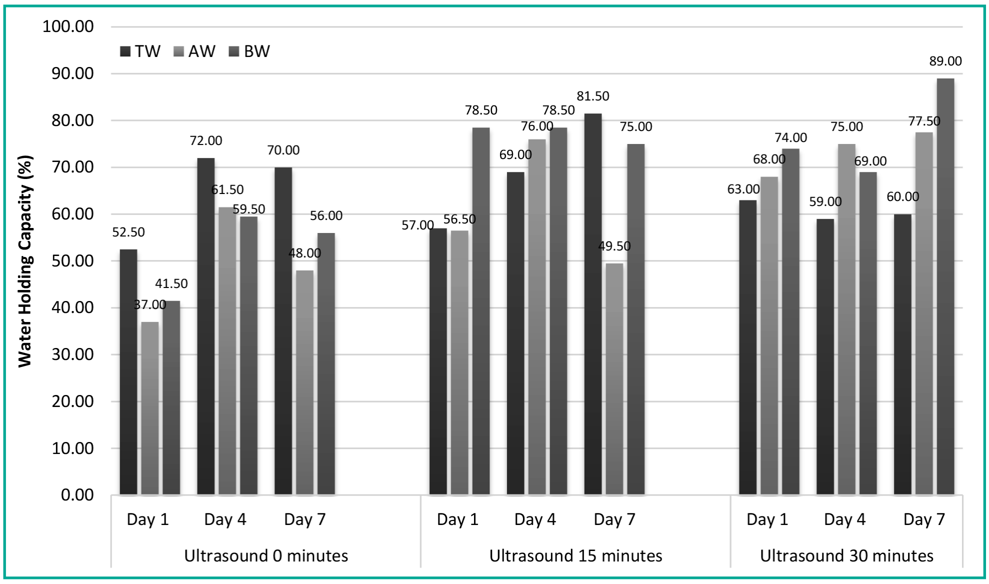

FIGURE 1: *Mean WHC values of samples during 7 day storage.*

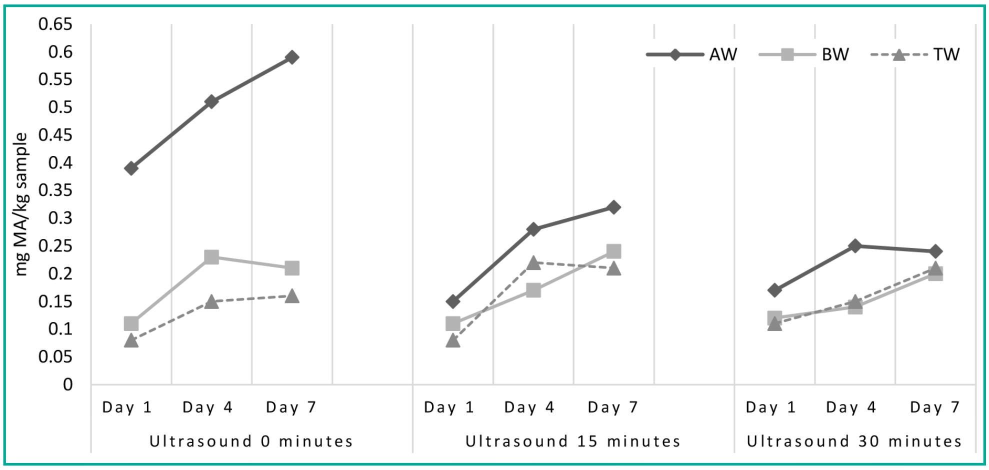

FIGURE 2: *Mean TBA values of samples during 7 day storage.*

cated that the TBA values increased constantly during the storage. The initial mean TBA values ranged from 0.08 to 0.39 mg MA/kg. Ultrasound treatments significantly ($p<0.05$) decreased the TBA values of the samples. The results of the present study were similar to those of Lima et al. (2018) who found that ultrasound application decreased the TBA values of Italian salami. The samples treated with AW showed significantly higher TBA values compared to the groups of BW and control. The group of U0-AW had the highest TBA values in all of the storage days. This result was in accordance with that of Cichoski et al. (2019) of who put forth that the chicken breast samples treated with slightly acidic electrolyzed water had higher TBA values than the samples treated with water. This situation could be attributed to the oxidizing properties of HClO in acidic electrolyzed water (Winterbourn et al., 1992; Huang et al., 2008). However, some researchers indicated that acidic electrolyzed water treatment decreased the TBA values of the meat samples (Rahman et al., 2012; Xu et al., 2014).

Microbiological enumarations

Total psychrophilic bacteria, *Enterobacteriaceae, Pseudomonas* spp. and *Salmonella* spp., counts (log CFU/g) of the samples are given in Table 3 showing each group and each day of the storage. On the first day of treatments, the total psychrophilic bacteria counts of the chicken breast meats decreased from 5.89 and 5.78 log CFU/g to 5.62 and 4.41 log CFU/g after 15 min ultrasound application and the numbers of psychrophiles were recorded as 5.65 and 4.49 log CFU/g after 30 min ultrasound application within TW and AW, respectively. The psychrophilic bacteria numbers of the breast chicken meats within BW were obtained as 6.09 and 4.85 log CFU/g after 15 and 30 min ultrasound treatments while the counts were 5.01 log CFU/g in non-treated meats. Thus regarding the psychrophilic bacterial counts, 15 min ultrasound treatment generated positive influence for the samples in AW and TW whilst that effect was 30 min later for the chicken meat samples in BW on the first day of storage. In previous studies researchers reported that bacterial counts could not increase with increasing trend of ultrasound application time. Indeed Carrillo-Lopez et al. (2019) stated that the increase in the sonication time from 20 to 40 min increased the count of the psychrophilic bacteria while the mesophilic bacterial counts decreased with the increasing time of ultrasound treatment. On the other hand, the psychrophilic bacteria numbers increased throughout the refrigerated storage in all groups without any exceptions in parallel with the results of Piñon et al. (2018). Meanwhile, the lowest psychrophilic bacteria numbers amongst all were observed in U1-AW and U3-AW (7.52 and 7.67 log CFU/g, respectively) whereas the highest counts were in UO-TW and UO-BW (9.53 and 9.49 log CFU/g, respectively) at the end of the storage. This approximately 2 log CFU/g reduction in the psychrophilic bacterial counts is greater than that in the reports of Cichoski et al. (2019) who observed 0.76 log CFU/g reduction in the psychrophile numbers after the treatment that combined the application of US 25 kHz for 10 min and slightly acidic electrolyzed water (pH 6.0). These differences were attributed to the good effect of low pH electroliyzed water and the higher ultrasound treatment times used in the U1-AW and U3-AW groups.

Regarding the *Enterobacteriaceae* counts, no bacterial growth was detected after the treatments in the U1-AW, U3-TW and U3-AW groups immediately after ultrasonication whereas in untreated control samples within TE, AW and BW the *Enterobacteriaceae* numbers were in the range of 4.03-4.49 log CFU/g on the first day of study. Similar to our results, Haughton et al. (2012) detected no *Enterobacteriaceae* on sonicated poultry skin whilst they found 3.39 log CFU/g on untreated poultry skin. Our reduction numbers for *Enterobacteriaceae* were higher than those of previously reported by Cichoski et al. (2019) who stated about 0.8 and 1.0 log CFU/g reduction in only ultrasound treated meat samples (24 kHz, 10 min) and samples subjected to ultrasound and slightly acidic electrolyzed combination (pH 6.0 SAEW, 24 kHz, 10 min), respectively. These differences may be due to the lower pH of waters and greater ultrasound duration applied in our sample groups. On the other side, the Enterobacteriaceae numbers displayed an increment throughout the cold storage in contrast with the findings of Carrillo-Lopez et al. (2019) who reported a reduction in coliform bacteria during the cold storage. Similar behaviours to ours were observed by Caraveo et al. (2015) who stated that the coliform bacteria counts constantly improved with time during 10 days of storage in both untreated and the sonicated beef samples. According to our results, on the last day of storage the Enterobacteriaceae numbers remained lower in the U1-AW and U3-AW groups amongst all samples (with 5.96 and 5.92 log CFU/g). Thus, taking into account these behaviours provided by 15 or 30 min. ultrasound treatment combined with AW, this combination is desirable to control and repress the *Enterobacteriaceae* counts in chicken breast meats.

In our study, *Pseudomonas* spp. counts were not detected in the U1-AW, U3-TW U3-AW, and U1-BW groups immediately after ultrasound treatment whereas in U0 groups in electrolyzed and tap waters, they ranged from

TABLE 3: *Effects of different water types and ultrasound treatment on total psychrophilic bacteria, Enterobacteriaceae, Pseudomonas spp. and Salmonella spp. counts (Log CFU/g) on chicken breast meats during storage at 4 °C for 7 days.*

Treatments		Total psychrophilic bacteria			*Enterobacteriaceae*			*Pseudomonas* spp.			*Salmonella* spp.		
		Day 1	Day 4	Day 7	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
UO	TW	5.89 ± 0.06^{Cb}	8.51 ± 0.01^{Ba}	9.53 ± 0.01^{Aa}	4.49 ± 0.05^{Cb}	4.63 ± 0.05^{Be}	7.35 ± 0.03^{Ab}	4.11 ± 0.02^{Ca}	4.69 ± 0.03^{Bb}	7.29 ± 0.04^{Ac}	3.92 ± 0.02^{Cb}	5.61 ± 0.03^{Bb}	7.72 ± 0.06^{Ab}
	AW	5.78 ± 0.01^{Cc}	7.36 ± 0.03^{Be}	8.26 ± 0.09^{Af}	4.64 ± 0.03^{Ba}	4.65 ± 0.07^{Be}	6.19 ± 0.04^{Ag}	3.49 ± 0.07^{Cc}	3.88 ± 0.04^{Be}	6.03 ± 0.05^{Ae}	3.41 ± 0.05^{Cd}	5.38 ± 0.07^{Bd}	6.33 ± 0.05^{Ae}
	BW	5.01 ± 0.01^{Ce}	8.52 ± 0.04^{Ba}	9.49 ± 0.02^{Aa}	4.03 ± 0.02^{Cd}	4.91 ± 0.02^{Bd}	6.41 ± 0.09^{Ae}	3.79 ± 0.05^{Cb}	4.68 ± 0.09^{Bb}	7.34 ± 0.11^{Ab}	3.69 ± 0.03^{Cc}	5.14 ± 0.02^{Be}	6.31 ± 0.03^{Ae}
U1	TW	5.62 ± 0.04^{Cd}	7.33 ± 0.02^{Be}	8.31 ± 0.01^{Ae}	3.88 ± 0.02^{Ce}	4.26 ± 0.08^{Bf}	6.92 ± 0.02^{Ad}	3.05 ± 0.02^{Cd}	3.44 ± 0.08^{Bf}	6.01 ± 0.08^{Ae}	3.13 ± 0.08^{Cf}	4.83 ± 0.06^{Bf}	7.46 ± 0.05^{Ac}
	AW	4.41 ± 0.02^{Ch}	6.54 ± 0.06^{Bg}	7.52 ± 0.07^{Ah}	ndg	3.71 ± 0.03^{Bg}	5.96 ± 0.05^{Ah}	ndg	ndg	5.71 ± 0.04^{Ag}	ndg	3.94 ± 0.09^{Bh}	5.13 ± 0.12^{Ag}
	BW	6.09 ± 0.01^{Ca}	7.50 ± 0.03^{Bd}	8.36 ± 0.09^{Ad}	4.38 ± 0.04^{Cc}	5.29 ± 0.06^{Bc}	6.36 ± 0.04^{Af}	ndg	4.41 ± 0.14^{Bd}	5.93 ± 0.05^{Af}	4.64 ± 0.02^{Ca}	5.76 ± 0.05^{Ba}	7.82 ± 0.08^{Aa}
U3	TW	5.65 ± 0.04^{Cd}	8.11 ± 0.05^{Bc}	9.28 ± 0.07^{Ac}	ndg	5.49 ± 0.07^{Ba}	7.22 ± 0.09^{Ac}	ndg	4.89 ± 0.07^{Ba}	7.41 ± 0.07^{Aa}	3.25 ± 0.11^{Ce}	5.55 ± 0.04^{Bc}	7.49 ± 0.07^{Ac}
	AW	4.49 ± 0.01^{Cg}	6.73 ± 0.03^{Bf}	7.67 ± 0.11^{Ag}	ndg	3.74 ± 0.01^{Bg}	5.92 ± 0.07^{Ai}	ndg	3.01 ± 0.03^{Bg}	5.64 ± 0.03^{Ah}	ndg	4.74 ± 0.09^{Bg}	6.02 ± 0.09^{Af}
	BW	4.85 ± 0.02^{Cf}	8.41 ± 0.02^{Bb}	9.41 ± 0.03^{Ab}	4.68 ± 0.01^{Ca}	5.39 ± 0.04^{Bb}	7.49 ± 0.04^{Aa}	4.09 ± 0.04^{Ca}	4.64 ± 0.06^{Bc}	7.11 ± 0.04^{Ad}	2.96 ± 0.06^{Cg}	5.41 ± 0.03^{Bd}	7.08 ± 0.04^{Ad}

Within the same row, values with different uppercase superscript letters indicate significant differences ($p < 0.05$) for each different microbial criteria. Within the same column, values with different lowercase superscript letters indicate significant differences ($p < 0.05$). ndg: No detectable growth. Water Treatments; TW: tap water, AW: acidic water, BW: basic water. Ultrasound Applications; U0: 0 min, U1: 15 min, U3: 30 min.

3.49 to 4.11 log CFU/g. Interestingly, no *Pseudomonas* spp. was found in the U1-AW groups on the fourth day of storage. On the last day of cold storage, the least numbers of Pseudomonas spp. were displayed in groups of U3-AW and U1-AW with 5.64 and 5.71 log CFU/g as in total psychrophilic bacteria and *Enterobacteriaceae*. A study conducted by Runyan et al. (2006) reported that ultrasonication enhanced the permeability of the outer membrane of *P. aeruginosa*. Therewithal, Haughton et al. (2012) and Carrillo-Lopez et al. (2019) notified that gram negative bacteria such as *Pseudomonas, Salmonella* and *Escherichia* were more sensitive to ultrasound than gram positive bacteria. Overall, our experimental data suggested that the combination of 15 or 30 min. ultrasound and acidic electrolyzed water (pH 2.1) could be effective in controlling *Pseudomonas* spp. especially in short-term cold storage of chicken breast meats.

For *Salmonella* spp., no growth was seen right after ultrasound application in the U1-AW and U3-AW groups whilst in untreated samples within TE, AW and BW *Salmonella* spp. the numbers were in the range of 3.41–3.92 log CFU/g on the first day of study. Subsequently, in all groups of the chicken breast meats the counts of *Salmonella* spp. gradually augmented towards the last day of cold storage. At the end of the storage, the least *Salmonella* spp. counts were determined in U1-AW group meats with 5.13 log CFU/g similar to the other microbiological parameters, however in U0-AW and U0-TW groups these numbers were 6.33 and 7.72 log CFU/g. Hence, it was clear that AW had limited effect on controlling *Salmonella* spp. counts alone, but when combined with a ultrasound treatment for 15 min, about 2.50 log CFU/g reduction occurred in the chicken breast meats on the last day of cold storage. Previously, Bi et al. (2020) revealed that *S. typhimurium* was inactivated by ultrasound and the numbers decreased about 2.23 log CFU/g during the 20 min treatment, however no significant changes were observed during this 30 min treatment just like in our findings. Similarly, São José et al. (2014) reported that the *Salmonella* cells sensitive to ultrasound might be inactivated instantly but they became activated again within longer treatment durations.

Textural assesment

Mean MORSF and MORSE values of raw and cooked chicken breast samples are given in Figure 3. While ultrasound applications significantly ($p<0.05$) affected the MORSF and MORSE values of raw and cooked chicken breast meats, electrolyzed water did not affect the textural parameters of the samples ($p>0.05$). As the durations of ultrasound got extended, the samples' tenderness increased. Samples sonicated for 30 min (U3) had lower MORSF and MORSE values. 15 min of ultrasound application improved the textural parameters of the samples compared to the control group (U0). The results of the present study are in agreement with those of Xiong et al. (2012) who investigated the texture of hen breast muscles that were treated with ultrasound (24 KHz for 15 s at 12 Wcm^{-2}), the lower shear force values were determined in the sonicated samples. Another study similar to this showed that after the application of ultrasound (24 kHz, 12 Wcm^{-2} for 4 min), tenderness of the bovine muscles increased (Jayasooriya et al., 2007). Xiong et al. (2020) reported that ultrasound application (20 KHz, 300 W, min sonication) combined with sodium bicarbonate marination had greater impact on the improvement of tenderization of the chicken breast meat samples than control (wet curing-ultrasound free). Shi et al. (2020) indicated that ultrasound (intensity of 15.6 W/cm^2 and 5 min) sonication combined with potassium alginate tenderized an old chicken breast meat. On the other hand, cooking process decreased the tenderness of the samples because cooked samples had higher MORSF and MORSE values than raw samples. According to this, we could say that heat-denaturation of myofibrillar proteins generally causes meats to toughen (Palka and Daun, 1999).

TABLE 4: *Average color parameters of sample groups.*

		*L**	*a**	*b**
Water Treatments	TW	51.79^b±2.48	1.98±0.77	4.02^a±1.20
	AW	54.34^a±2.00	1.37±0.65	1.56^b±1.87
	BW	51.47^b±1.59	1.72±0.65	2.16^b±0.98
Ultrasound Treatments	U0	52.44±1.20	1.65±0.80	2.26^b±2.12
	U1	53.06±3.51	1.57±0.58	3.41^a±1.60
	U3	52.11±1.98	1.85±0.72	2.09^b±1.35

[a–b] Means with no common superscripts are significantly different (*p<0.05)

Color parameters

L, a** and *b** values of the samples are shown in Table 4. *L** values of the samples decreased during the storage whereas *b** values increased. Ultrasound application had no significant ($p>0.05$) effect on *L** and *a** values while it affected significantly ($p<0.05$) *b** values of the samples. The U1 group had the highest mean *b** values of the samples. The present results were partly in agreement with the reports of Jayasooriya et al. (2007) who reported that ultrasound treatment had no important effect on the color parameters (*L*, a**, and *b**). Electrolyzed water treatment had a significant effect ($p<0.05$) on *L** and *b** values of the chicken breast meats whereas a* values had no significant ($p>0.05$) changes. The results of the present study are in agreement with those of Chaijan et al. (2005) and Li et al.

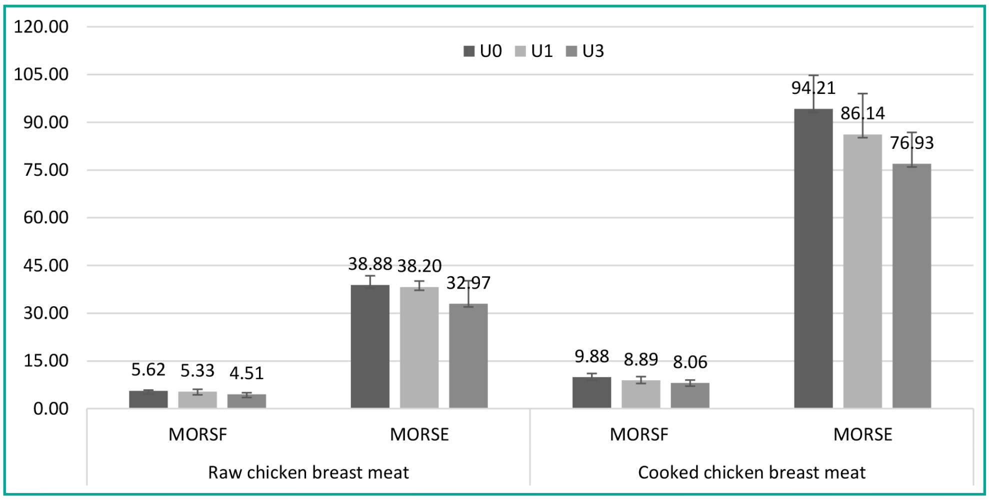

FIGURE 3: *Effect of ultrasound application on MORSF (N) and MORSE (N.mm) values of raw and cooked chicken breast meat samples.*

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(2011) who investigated the application of different types of electrolyzed water did not affect the red color of the loins.

Sensory evaluation

Figure 4 indicates the color, odor, tenderness and overall acceptance of the cooked chicken breast meat samples. Interaction of ultrasound application and electrolyzed water had an important effect ($p<0.05$) on tenderness and overall acceptance. The group of U1-BW was determined as the most tender sample by the panelists. As for the overall acceptance, U1-AW and x U1-BW interactions had the highest means. In agreement with our results, several previous studies reported that ultrasound application improved the perception of sensory attributes of ham (Barretto et al., 2018) and beef (Peña-Gonzalez et al., 2019).

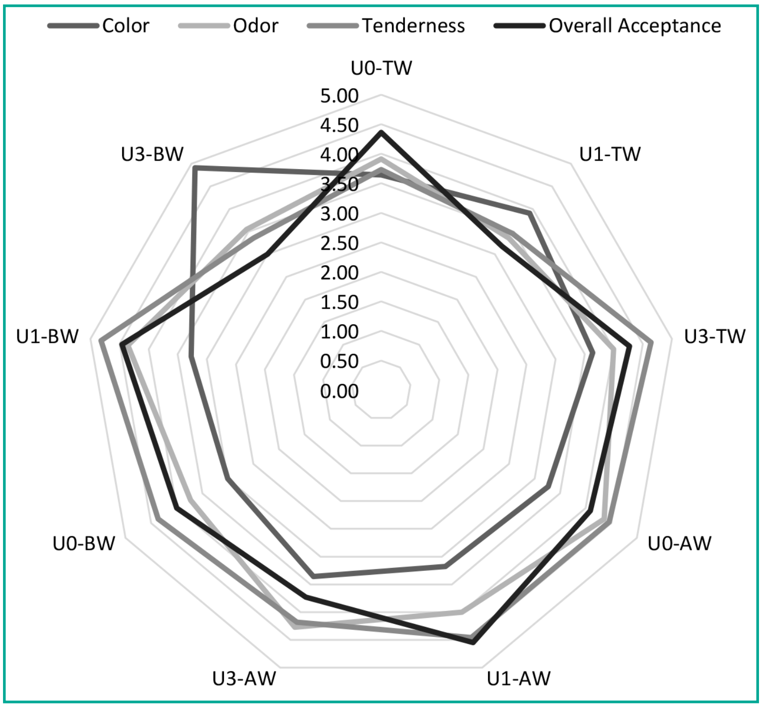

FIGURE 4: *Mean scores of color, odor, tenderness and overall acceptance of cooked chicken breast meat samples.*

Conclusions

Ultrasound application increased the water holding capacity of the samples. Based on microbiological data the combination of ultrasound treatment for 15 or 30 min and acidic electrolyzed water (pH 2.1) significantly decreased the number of psychrophiles, *Enterobacteriaceae, Pseudomonas* spp. and *Salmonella* spp. of the chicken breast meats. However, acidic water caused lipid oxidation in the samples. Dipping in electrolyzed water did not have a significant effect on MORSF nor MORSE, while ultrasound improved the texture of the chicken breast meats.

Conflict of interest

We have no conflict of interest to declare.

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Address of corresponding author:
Ali Samet Babaoğlu
Department of Food Engineering
Agriculture Faculty
Selçuk University
42050, Konya
Turkey
asmtbb@gmail.com